

**Amendments to the Specification:**

The paragraph starting at page 16, line 24 has been amended as follows:

Figure 1 shows restriction maps of clones used to isolate the pathogenic region and maps of the two pathogenic variants pMH32 and pMH41, in accordance with a preferred embodiment of the present invention; and where:

(A) Is the pADAP *Hind*III clone pGLA-20 showing locations of the pGLA-20 mutations – 10, -13, and 35, which when recombined back into pADAP and bioassayed against grass grub, result in either a pathogenic phenotype, shown by full flag, or a healthy but non-feeding phenotype indicated by half filled flag. Map of pBG35 showing relative position of pGLA-20-35 mutation and the location of the 2.2kb *Eco*Ri used as a probe to screen the pADAP *Bam*HI library; and

(B) Illustrated restriction enzyme maps of the pathogenic clones pMH32 and pMH41, area of deletion is indicated by  $\Delta$ .

 pBR322 vector DNA;

 pLAFR3 vector DNA.

Restriction enzymes are abbreviated as follows: B, *Bam*HI, Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; and X, *Xba*I.

The paragraph starting at page 17, line 2 has been amended as follows:

Figure 2 shows deletion derivatives used in the study, restriction maps of the mutated constructs and recombinants, the phenotype of each mutation, the schematic diagram of the sequenced region, and a nucleotide sequence in accordance with a preferred embodiment of the present invention; and

(A) Which are Mini-Tn10 pACYC184 based deletion derivatives used in the study.

 is the pACYC184 vector,

$\Delta$  indicates deletion + pathogenic,

- loss of pathogenicity; and

(B) Illustrates restriction maps of the mutated constructs pBM32 and the pADK recombinants; and

(C) Where the phenotype of each mutant is indicated by flags.

Blocked flags indicates mutations that did not affect the disease process.

Open flags indicate mutations that abolish disease symptoms.

Half-filled flags denote mutations that abolish visual disease symptoms but are unable to feed.

\* indicates pADK mutations obtained by Grkovic et al. (1995).

Restriction enzymes are abbreviated as follows: B, *Bam*HI, Bg, *Bg*II, E, *Eco*RI; H,

*Hind*III; and X, *Xba*I.

(D) Is a schematic diagram of the sequenced region, where:



Denotes sequenced region.

Arrows indicate ORFs and their direction



region homologous to spvB ... location of repeat.

(E) Is a nucleotide sequence of the 5 times 12bp repeat and the palindrome.

Restriction enzymes are abbreviated as follows: B, *Bam*HI, Bg, *Bg*II; E, *Eco*RI; H, *Hind*III; and X, *Xba*I.

The paragraph starting at page 17, line 5 has been amended as follows:

Figure 3 shows hydrophobicity plots of SepC and its closest homologue TccC, in accordance with a preferred embodiment of the present invention; and the scale is disproportional to size and has a scanning window of 17 amino-acid residues.

The paragraph starting at page 17, line 8 as been amended as follows:

Figure 4 shows the comparison of protein sequences of the SepA and *P. luminescens* toxins, TcdA, TcaB (SEQ ID NO:7) and TccB (SEQ ID NO:8) Putative RGD motif is boxed, plus the site of proteolytic cleavage is illustrated, in accordance with a preferred

embodiment of the present invention; and The site of proteolytic cleavage is reported by Bowen et al. (1998) (Residue 1933 of TcdA) is indicated by an arrow.

The paragraph starting at page 17, line 12 has been amended as follows:

Figure 5 shows the comparison of protein sequences of the SepC (SEQ ID NO:9) and *P. luminescens* toxin TccC, in accordance with a preferred embodiment of the present invention; and

The paragraph starting at page 17, line 20 has been amended as follows:

Figure 1 shows restriction maps of clones used to isolate the pathogenic region and maps of the two pathogenic variants pMH32 and pMH41, where:

(A) Is the pADAP *Hind*III clone pGLA 20 showing locations of the pGLA 20 mutations 10, 13, and 35, which when recombined back into pADAP and bioassayed against grass grub, result in either a pathogenic phenotype, shown by full flag, or a healthy but non-feeding phenotype indicated by half filled flag. Map of pBG35 showing relative position of pGLA 20 35 mutation and the location of the 2.2kb *Eco*Ri used as a probe to screen the pADAP *Bam*HI library; and

(B) Illustrated restriction enzyme maps of the pathogenic clones pMH32 and pMH41, area of deletion is indicated by  $\Delta$ .

 — pBR322 vector DNA;

 — pLAFR3 vector DNA.

Restriction enzymes are abbreviated as follows: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; and X, *Xba*I.

The paragraph starting at page 18, line 12 has been amended as follows:

Figure 2 shows:

(A) Which are Mini-Tn10-pACYC184 based deletion derivatives used in the study.

 — is the pACYC184 vector.

~~△~~—indicates deletion + pathogenic;

~~—~~—loss of pathogenicity; and

~~(B) Illustrates restriction maps of the mutated constructs pBM32 and the pADK recombinants; and~~

~~(C) Where the phenotype of each mutant is indicated by flags.~~

~~Blocked flags indicates mutations that did not affect the disease process.~~

~~Open flags indicate mutations that abolish disease symptoms.~~

~~Half filled flags denote mutations that abolish visual disease symptoms but are unable to feed.~~

~~\* indicates pADK mutations obtained by Grkovic et al. (1995).~~

~~Restriction enzymes are abbreviated as follows: B, *Bam*HI, Bg, *Bgl*II, E, *Eco*RI; H,~~

~~*Hind*III; and X, *Xba*I.~~

~~(D) Is a schematic diagram of the sequenced region, where:~~

 — Denotes sequenced region.

Arrows indicate ORFs and their direction

 — region homologous to *spvB* ... location of repeat.

~~(E) Is a nucleotide sequence of the 5 times 12bp repeat and the palindrome.~~

~~Restriction enzymes are abbreviated as follows: B, *Bam*HI, Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; and X, *Xba*I.~~

The paragraph starting at page 19, line 13 has been amended as follows:

~~In Figure 3 hydrophobicity plots of SepC and its closest homologue TeeC are shown. The scale is disproportional to size and has a scanning window of 17 amino acid residues.~~

The paragraph starting at page 19, line 15 has been amended as follows:

~~Figure 4 shows the comparison of protein sequences of the SepA and *P. luminescens* toxins, TedA, TeaB and TeeB. Putative RGD motif is boxed. The site of proteolytic cleavage is reported by Bowen et al. (1998) (Residue 1933 of TedA) is indicated by an arrow.~~

The paragraph starting at page 19, line 19 has been amended as follows:

~~Figure 5 shows the comparison of protein sequences of the SepC and *P. luminescens* toxin TeeC; and Figure 6 shows the plasmid pADAP.~~

The paragraph starting at page 22, line 5 has been amended as follows:

A 9-kb *Bam*HI–*Eco*RI fragment derived from the pBM32-8 mutation (Fig 2b) and the 8kb *Hind*III fragment of pBM32 were separately cloned into the appropriate site of the deletion factory plasmid pDELTA1. Deletions were generated using the Deletion factory™ system (GIBCO BRL, MD, USA), as outlined in the manufacturer's instructions. To identify the precise location of mini-*Tn*10 mutations, the peripheral mini-*Tn*10 *Bam*HI sites were used in conjunction with the *Bam*HI sites of the pathogenic region to subclone the mini-*Tn*10 flanking regions into either pACYC184 or pUC19. Sequences were generated using the mini-*Tn*10 specific primer 5'ATGACAAGATGTGTATCCACC3' (SEQ ID NO:10) (Kleckner et al. 1991).

The paragraph starting at page 29, line 3 has been amended as follows:

The hydropathicity profiles of each of the Sep proteins was examined using the Kyte and Doolittle algorithm (Kyte and Doolittle, 1982) and compared to the relevant *P. luminescens* homologues. None of the Sep proteins contained a positively charged amino terminus followed by a hydrophobic region, characteristic of a signal sequence (Giersch, 1989). The profiles of SepA, TcbA and TcdA were very similar (data not shown) and each exhibited a steep hydrophilic peak at the carboxyl terminus (residues 2055-2061 of SepA), specifically the protein sequence RRRRE (SEQ ID NO:11) (Fig. 4). Although both SepB and TcaC shared similarity to the *Salmonella* virulence protein SpvB, the amino-termini of SepB and TcaC were hydrophilic as opposed to the hydrophobic nature of SpvB. The profile of SepC and its *Photobacterium* counterpart TccC differed in that SepC had a slightly hydrophilic amino-terminus, whereas TccC lacked a hydrophilic amino-terminus and had a significantly hydrophobic carboxyl terminus from amino-acid residue 717 onwards (Fig. 3).

**Amendments to the Sequence Listing:**

Please replace the Sequence Listing with the revised Sequence Listing.